PHYLOGENETIC RELATIONSHIPS AMONG GENERA OF THE SUBTRIBE ONCIDIINAE (EPIDENDROIDEAE: ORCHIDACEAE) AND A NEW GENUS: *SANTANDERELLA*

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ABSTRACT

Santanderella, a new genus of orchids from Colombia with the type species, *Santanderella amado-rinconiana*, related to *Macroclinium* and *Notylia,* is analyzed both at the phenotypic and genotypic levels. Phylogenetic trees related to genomic *matK-trnK* and *ITS*1-5.8S-*ITS2* sequences are presented to support the proposal of a new genus. *Phytologia 93(3):388-406 (December 1, 2011).*

KEY WORDS: Orchidaceae, Oncidiinae, *Santanderella,* Colombia, *matK-trnK*, *ITS*1-5.8S-*ITS2*.

An orchid plant belonging to the subtribe Oncidiinae (sensu R. Dressler, 1981) and showing affinity with the genera Notylia Lindl. and Macroclinium Barb. Rodr., was collected by Jonathan Amado in Floridablanca, Santander, Colombia, and reported by Orlando Rincón in 2009 (Figure 1A).

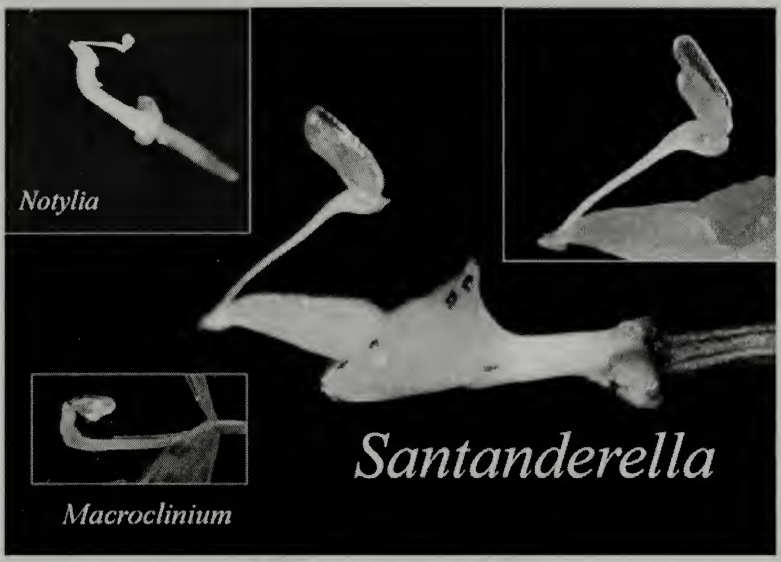

Figure 1A. *Santanderella amado-rinconiana* P. Ortiz. Comparison of the columns of the three related genera: *Notylia*, *Macroclinium* and *Santanderella*. Notice the peculiar shape of the column and the pollinia of *Santanderella*.

A number of characters of this specimen showed affinity with species of *Notylia*: the epiphytic, caespitose plant with unifoliate pseudobulbs and conduplicate leaves, the many-flowered racemose inflorescence, the rather large dorsal anther, the two pollinia with a thin and elongated stipe, and the ventral stigma as a narrow, longitudinal slit. Many of these characters are also found in the genus *Macroclinium*. But at the same time, the structure of the column and the pollinia, in addition to the characters of the sepals and petals, and especially of the lip, presented marked differences when compared to those of the close genera.

The plant we are dealing with has flowers that do not open fully (which seems to be a general condition of all the plants of this species seen by the collectors), with narrow sepals and petals, and a lip

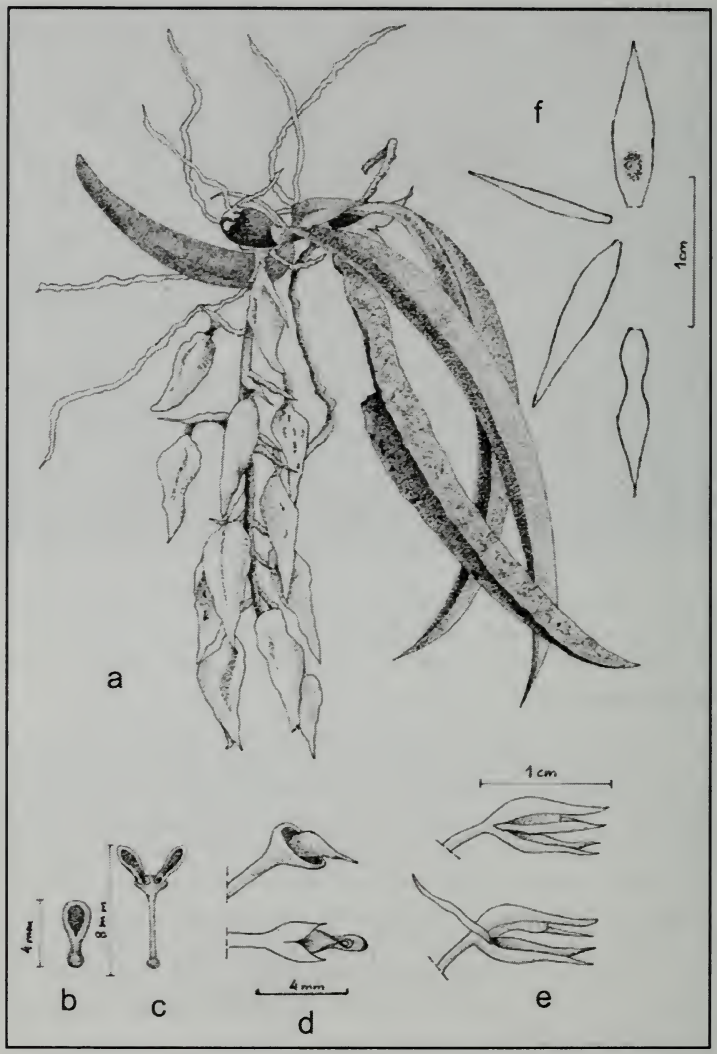

Figure 1B. *Santanderella amado-rinconiana:* **a-** Plant; **b-** Anther; **c-** two elongated, laminar, concave and yellow pollinia, affixed to a stipe with a triangular apex, then thin, 4 mm long; **d-** column, side and ventral views; **e-** Flower; **f-** Sepals, petals and lip.

that is different from all the "notyliiform" lips so far seen. It is very narrow, with a pair of small rounded lobules at the base, then turns narrow again, and then widens a little, with a subacute apex. There is no callus. It reminds the flowers of *Macroclinium*. The column is relatively short, with a basal part terete, and it widens apically into two obtuse, irregular wings, which ventrally merge together forming an acute angle. There is a clinandrium with rather high walls and inside the cavity the rostellum stands out, which is thick and high at the base and extends forward into a sharp point. The column does not bend backwards as in most *Notylia* species, but is rather straight. On the ventral part of the rostellum the stigma can be seen as a narrow slit. The anther is similar to those of *Notylia* and *Macroclinium*. But the pollinia are most remarkable. There are two pollinia, as in all of the Oncidiinae, but unlike the pollinia of *Notylia* and *Macroclinium*, they are quite large and elongated, flattened and concave. This type of pollinia, as far as we know, is not found in any species of *Notylia* or *Macroclinium*. The Oncidiinae genera close to *Notylia* have been defined and characterized in different ways, as can be seen in the study published by Pupulin (1997), to which we refer for further information. According to his study, the main difference between *Notylia* and *Macroclinium* lies in the shape of the leaves: dorso-ventrally flattened (*Notylia*) vs. laterally flattened (*Macrocliniuum*). The leaves of *Santanderella* are dorso-ventrally flattened, but are V-shaped.

We came to the conclusion that a new genus had to be established to accomodate this new species and so, on the basis of the phenotypic analysis, it was published in *Orquideología* (Medellín) 27(2): 167-178, 2011 (sub 2010) (Ortiz, 2011). Although establishing monotypic genera is not ideal, we cannot stretch out the limits of the genera to force incongruous elements into established genera. On the other side, this is not the only monotypic genus within this group (equally monotypic are *Notyliopsis*, *Sarmenticola*, *Chelyorchis, Hintonella, Hofmeisterella,* and *Schunkea*).

We then proceeded to a molecular analysis to determine the phylogenetic affinities of this eventual new genus with different orchids, which have already been reported by us and others in GenBank including: *Santanderella amado-rinconiana, Macroclinium xiphophorum, Notylia incurva, Notyliopsis beatricis, Oncidium*

(Trichocentrum) lanceanum, Oncidium ornithorhynchum, Oncidium cultratum, Oncidium (Otoglossum) globuliferum, Oncidium fuscatum, Brassia sp., Macradenia brassavolae, Trichocentrum pulchrum, Oliveriana ortizii, Telipogon nervosus, Oncidium (Trichocentrum) carthagenense.[1]

In the present study, we present the phylogeny of the new genus *Santanderella amado-rinconiana* using plastid and nuclear markers (*matK-trnK* and *ITS*1-5.8S-*ITS2* sequences) and evaluate the classification systems previously proposed by Ortiz (2011), based on morphological characters.

MATERIALS AND METHODS

Taxon sampling

We first sampled 15 currently recognized species of the subtribe *Oncidiinae* (Pridgeon, 2009) available on local crops that were not previously reported in GenBank and performed phylogenetic analysis comparing these genera with *Santanderella amado-rinconiana* (Table 1). We only included *matK-trnK* and *ITS*1-5.8S-*ITS2* sequences of the closest taxa, according to the most recent classification of the family (Chase et al, 2005), as can be seen on Table 2. The comparing genera thus included the following: *Macroclinium, Notylia* and *Macradenia. Notyliopsis* was selected as an outgroup, following the principles stated by Felsenstein (1985) and Swofford (2002).

DNA extraction

Plant tissues were dried using silica gel and stored at 70°C (Chase and Hills, 1991). DNA was extracted using a modified CTAB protocol (Doyle and Doyle, 1987). Approximately 0.25 g of green tissue was ground under a mortar and was transferred to a 1.5ml eppendorf tube. Seven hundred microliters (µl) of hot (65°C) CTAB buffer (0.02 M EDTA, 1.4M NaCl, 0.1 M Tris pH 8.0, 2% CTAB, 0.7%

[1] For an alternative nomenclature used recently by other authors (included here in parenthesis), refer to the Kew webpage "World Checklist of Selected Plant Families", in: apps.kew.org/wcsp/home.do

v/v DTT, 2% soluble PVP) was added. The slurry was incubated at 65°C for 30 min with occasional shaking, followed by extraction with an equal volume of chloroform-isoamyl alcohol (24:1). Phases were separated by centrifugation for 10 min at 16,000g. The aqueous phase was removed and reextracted with chloroform-isoamyl alcohol. The aqueous phase was removed again and two hundred ninety one µl of isopropanol and forty µl of ammonium acetate 7.5 M were added, gently mixed, and incubated at -20°C overnight. The DNA was pelleted at 20,000g for 5min. The pellet was washed briefly in 76% ethanol/ 0.01 M sodium acetate and then centrifuged for 5 min. The supernatant was removed; the pellet was air-dried and resuspended in100 µl of TE Buffer (10m MTris, pH 8, 0,1 mM EDTA).

DNA amplification

When necessary, DNA was cleaned using a Pure Link PCR® purification kit (Invitrogen, USA) according to manufacturer's instructions. A 1482 bp fragment from the 30 end of the *matK-trnK* gene was amplified using primers 19F and 556R (Table 3) in the PCR. Each PCR had a final volume of 100 µl and contained 10–20 ng of genomic DNA, 200uM each dATP, dCTP, dTTP and dGTP, 2.5 mM MgCl2, 0.5 uM forward (19F - 390F) and reverse (556R and 1326R) primers, 1.25 U Taq DNA polymerase GO (Promega, USA) and 5 X of buffer green of Taq DNA polymerase GO buffer (Promega, USA). Cycling conditions were: initial melting at 94 °C for 5 min; 39 cycles of 94°C for 1min, 48.6°C for 1min, 72°C for 2 min; final extension was set at 72 °C for 15 min.

The amplification of the nuclear internal transcribed spacer (*ITS*) region sequences (also defined as *ITS*1-5.8S-*ITS*2) on the following species was reported by ourselves on GeneBank: *Notylia incurva*, *Notyliopsis beatricis*, *Santanderella amado-rinconiana* and *Macroclinium xiphophorum*. Fifteen additional *ITS* sequences (7 *Macroclinium sp.* and 8 *Notylia sp.*) were included in our phylogenetic analysis. The amplification of the *ITS*1-5.8S-*ITS2* region was conducted in a polymerase chain reaction (PCR) with the primer sequences proposed by Sun (1994) (Table 4). The reagent PCR volume of 100µl reactions contained: 5x Go taq Promega Buffer, 10 µl of bovine serum albumine (BSA), 25mM MgCl2, 10 mM of each primer, 2 µl of Promega Go Taq (5U/µl), 10mM of dNTPs, 4 µl of dimethyl sulfoxide

(DMSO), genomic DNA (20 ng/µl) and 58 µl of water. The PCR protocol included: one first step of initial denaturation 5 minutes (95°C), 30 cycles of 1 min denaturation (94°C), 1 min annealing (54°C), and 2 min, 30 s elongation (72°C), with two additional seconds elongation per cycle and a final elongation step of 7 minutes (72°C).

DNA sequencing

PCR products were purified using a QIAquick DNA Cleanup System® (Qiagen, Germany) and sequenced using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit® (Applied Biosystems, USA), following the recommendations of the manufacturer. The sequencing products were analyzed by an ABI 3100 Avant Sequencer® (Applied Biosystems, USA). The sequences were assembled in Sequencher 3.0 (Gene Codes, Ann Arbor, Michigan, USA) and aligned manually in MacClade v. 4.08 (Maddison & Maddison, 2005). Gaps were coded separately and excluded from the analyses. Regions with ambiguous alignments were also excluded.

Phylogenetic analysis

Maximum parsimony (MP) and maximum likelihood (ML) analyses were performed in PAUP*, version 4.0b10 (Swofford, 2002). MP and ML heuristic searches used 1,000 replicates of random taxon stepwise-addition (retaining 20 trees at each replicate), tree bisection reconnection (TBR) branch swapping, and equal weighting of all characters. For ML searches, the best-fit model of nucleotide substitution and model parameters were determined for *matK-trnK* and for *ITS* using ModelTest 3.04 (Posada & Crandall, 1998); F81+I+G and K81uf+I+G were respectively identified as the most appropriate models of evolution for each of these data sets. Support was accessed with non-parametric bootstrapping; heuristic searches with 1000 replicates for MP and 100 replicates for ML were conducted using the same parameters as described above. Clades with bootstrap support of 50–74% were considered weakly supported, 75–89%, moderately supported, and 90–100%, strongly supported.

RESULTS

The data sets for *ITS* and *matK-trnK* sequences presented different levels of variation and contained varied amounts of indels, as can be seen on Table 5. Specific *matK-trnK* gene sequences were generated for the new genus *Santanderella amado-rinconiana*, and for *Macroclinium xiphophorum, Notylia incurva, Notyliopsis beatricis, Oncidium (Trichocentrum) lanceanum, Oncidium ornithorhynchum, Oncidium cultratum, Oncidium (Otoglossum) globuliferum, Oncidium fuscatum, Brassia sp, Macradenia brassavolae, Trichocentrum pulchrum, Oliveriana ortizii, Telipogon nervosus, Oncidium (Trichocentrum) carthagenense.* Sequences are available in GenBank (accession numbers provided in Table 1). Data in the combined data set (*ITS* and *matK-trnK*) contained several small gaps (up to 20 bp in length) and an aligned matrix with 1611 characters. MP analysis for this marker resulted in 6478 trees of 749 steps with a CI of 0.52 and a RI of 0.73; overall, 17.9% of the sites included in the analyses were informative (Table 5).

ITS sequences were obtained for *Santanderella amado-rinconiana, Notyliopsis beatricis, Macroclinium xiphophorum* and *Notylia incurva*. The corresponding MP search resulted in 3,414 trees of 179 steps (CI=0.65; RI=0.75). The aligned matrix resulted in 558 characters of which 7.9% were parsimony informative (Table 5). The ML search led to a single tree with -lnL = 1807.26573. The topologies obtained through the MP and ML analyses were congruent with respect to all strongly supported clades. The ILD (P=0.001) and Templeton tests (rival tree *ITS*, $p<0.0001$; rival tree plastid, p=0.34) suggested that the *matK-trnK* data set is incongruent with *ITS*. Furthermore, several contradictory relationships were found between the *matK-trnK* and *ITS* topologies (data not shown). Hence, *ITS* data sets were analyzed in combination with *matK-trnK* data sets through MP and ML analyses. Phylogenetic relationships among species were consistent in both ML and MP phylograms (Figures 2 and 3).

In the first step, *matK-trnK* and *ITS* sequences were used to perform a broader analysis on representatives of all Orchidaceae to test the monophyly of Oncidiinae, and also to explore their position within

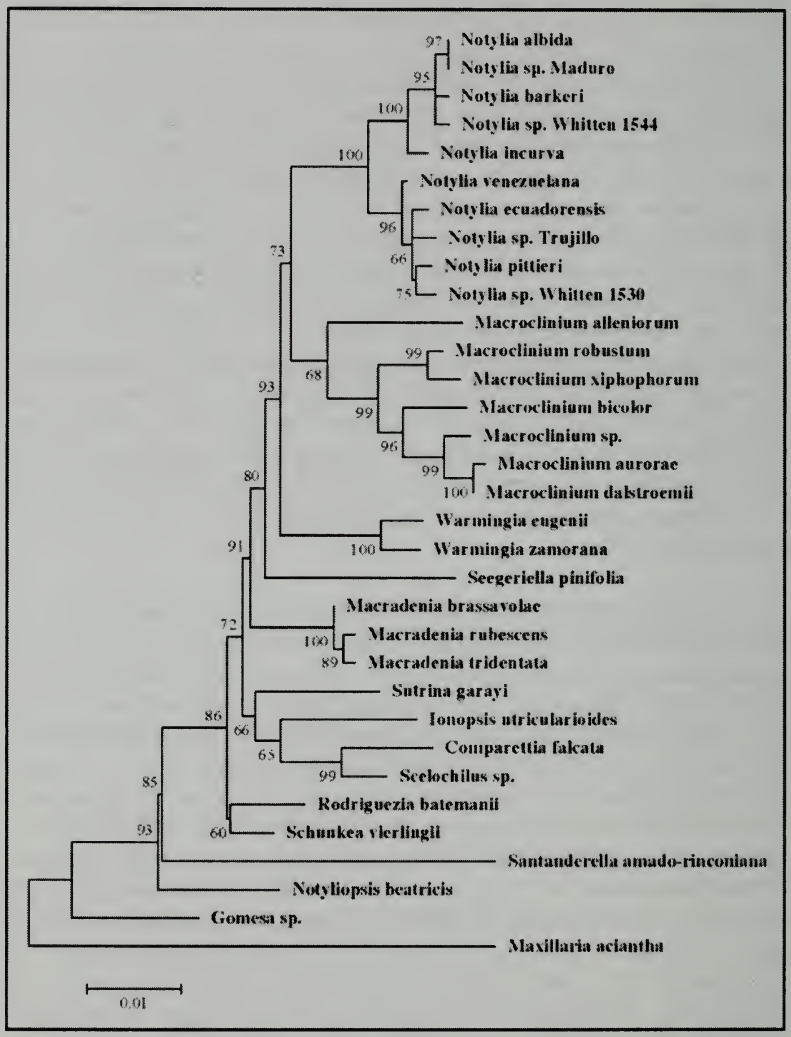

Figure 2. Maximum likelihood phylogram based on combined *matK-trnK* and *ITS* data.

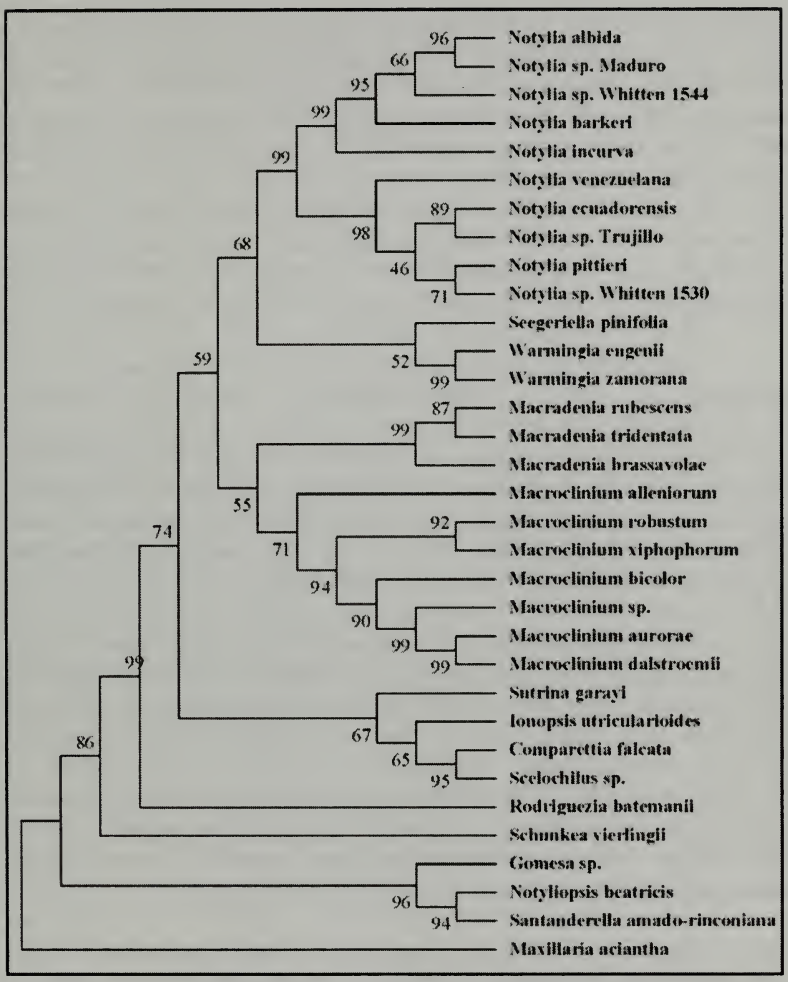

Figure 3. Maximum parsimony (MP) strict consensus topologies, combined analysis between *matK-trnK* and *ITS* sequences in the genera of the subtribe *Oncidiinae* close to *Santanderella.* Maximum parsimony bootstrap values are shown above branches.

the family performed on all the genera included in the phylogenetic three published by Chase (2005) (Table 1, Figures 2 and 3). Subsequently, more restricted analyses were performed in order to compare separately *Macroclinium*, *Notylia, Macradenia, Notyliopsis* and *Santanderella* based on their *ITS* 1-2 and *matK-trnK* sequences, then *Santanderella amado-riconiana* and *Notylia* were compared on their *ITS* and *matK-trnK* sequences and, finally, *Santanderella amado-riconiana* was compared to *Macroclinium* based on their *ITS*1-*ITS*2 and *matK-trnK* sequences (data not shown). Every phylogram confirmed the monophyly of the new genus *Santanderella amado-riconiana*.

Restricted analyses of both *matK-trnK* and *ITS* sequences were performed in order to compare separately *Santanderella amado-rinconiana* with each taxonomic subgroup. When *matK-trnK* sequences were compared within the genus *Notylia*, we found that *Notyliopsis beatricis*, *Notylia venezuelana* and *Santanderella amado-rinconiana* appear as outgroups. In contrast, when *matK-trnK* sequences were compared within the genus *Macroclinium*, only *Santanderella amado-rinconiana* appears as an outgroup (data not shown).

When only *matK-trnK* sequences of *Santanderella* were compared within a wider sample population which included *Macroclinium*, *Notylia, Macradenia* and *Notyliopsis,* no clear-cut distinction was found between species belonging to those genera. However, four species, namely *Santanderella amado-rinconiana, Notyliopsis beatricis, Macradenia brassavolae* and *Notylia sp.* appeared to correspond to outgroups in this phylogeny. In the central clades a *Macradenia* species appeared to be closely related to *Macroclinium chasei* and *Macroclinium alleniorum* (data not shown).

Moreover, when *ITS* sequences from *Santanderella* were compared within *Macroclinium*, *Notylia, Macradenia* and *Notyliopsis*, both *Santanderella amado-rinconiana* and *Notyliopsis beatricis* appeared as outgroups. When nuclear genetic markers were compared, a clearer distinction was found between species belonging to the genera *Notylia* and *Macroclinium* which now appear clearly monophyletic (data not shown).

Furthermore, we incorporated additional analysis with a combined sequences (ITS and matK-trnK) in a pooled analysis with the most related genera that were included in the phylogenetic three published by Chase (2005) based on *matK-trnK* sequences. We then selected *Maxilaria aciantha* as an outgroup, and we confirmed the particularity of two specific genera, namely *Santanderella amado-rinconiana* and *Notyliopsis beatricis*, as compared to the other species of the subtribe *Oncidiinae* belonging to *Macroclinium, Macradenia* and *Notylia*. These two apparently monophyletic genera appeared on an outside cluster in relation to other monophyletic genera in this phylogeny both by the ML and MP approaches (Figures 2 and 3).

DISCUSSION

In this study, we used one plastid molecular marker (*matK-trnK*) and a nuclear data set (*ITS*) to investigate phylogenetic relationships within the subtribe Oncidiinae and genera more closely associated to the new genus proposed as *Santanderella* (Ortiz, 2011). The *ITS*1-5.8S-*ITS*2 markers produced congruent topologies while *matK-trnK* topologies suggested a slightly different scenario than the one recovered with the nuclear data. In the following paragraphs, we discuss the results from phylogenetic analyses, differences between the *ITS* and plastid topologies, and the implications of this results for the systematics of the new genus *Santanderella*.

Literature of molecular systematics of orchids is growing as can be seen in previously published reports (Pridgeon et al, 2001; Salazar et al, 2009) and also on GenBank databases, where 4710 sequences belonging to Oncidiinae have been reported on 793 species belonging to 73 genera, including 15 new species reported by ourselves. The results revealed that neither *Macroclinium*, *Macradenia, Notylia* and *Notyliopsis* show molecular phylogenetic affinity with *Santanderella amado-rinconiana.* However, as we consider that molecular phylogenetic affinity to determine a taxonomic category has to include phenotypic considerations, we combined phenotypic and genotypic criteria for the description and classification of this new genus.

The molecular approach confirms our first impression based on phenotypic characters, as the specimen proposed as a new genus (Ortiz, 2011) appears indeed isolated on a different branch both by *matK-trnK* and *ITS* maximum parsimony strict consensus topologies, with bootstrap values over 90 and posterior probability values over 0.90. On this grounds, lumping *Santanderella amado-rinconiana*, and also *Macroclinium chasei* and *Macroclinium alleniorium*, or even the genera *Notylia*, *Notyliopsis*, *Macroclinium* and *Macradenia* as has been suggested as an ultimate option (F. Pupulin and M. Chase, personal communications), would seem inappropriate, specially if the studies based in morphological characters such as the one reported by Pupulin (1997) on the phylogeny of *Macroclinum* are taken into consideration. In this case, *Macroclinium chasei* appears linked only by a doted line to the main branch of this taxonomic group. Other genera in Oncidiinae are being subjected to taxonomic transfers (Chase and Whitten, 2011), while a word of caution has been proposed on further studies of phylogenetic delimitation in plants before a world-wide consensus is reached (Vanderpoorten and Shaw, 2010).

Nevertheless, our results strongly support our hypothesis of a new genus for *Santanderella amado-rinconiana*, as an option to clarify the diversity of orchids within the Oncidiinae subgroup, both at the phenotypic and genotypic levels. We have demonstrated a clear genotypic and phenotypic separation of *Santanderella* against both *Notylia* and *Macroclinium*, further supporting the validity and specificity of *Santanderella* as based on its long branch (reflecting its clear morphological identity) compared to the other segregate genera sampled.

As stated in the introduction, establishing monotypic genera is not ideal. However, as we cannot stretch out the limits of the genera to force incongruous phenotypic elements into established genera, we also conclude that the presence of monotypic genus within this group (*Notyliopsis*, *Sarmenticola*, *Chelyorchis, Hintonella, Hofmeisterella, Schunkea* and *Santanderella*) implicates the existence of multiple segregate (most likely oligospecific) genera in the vicinity of the *Notylia* and *Macroclinium* "clade". The need to accept a new genus is thus based on its clear genetic differentiation from these segregate genera, but also because of its discrete and patent morphological

identity, worthy of constituting a new generic entity. As stated by some researchers (Santiago Madriñán, personal communication), this is the case of numerous examples in systematics, where speciose monophyletic groups characterized by clear autoapomorphies are accompanied by a grade of oligospecific groups –each with its own autoapomorphy–, which cannot be included in the larger groups diluting their identity as to the characters that allow their recognition, and which cannot be placed within a single entity due to their non monophyly.

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Table 1. Sampling of taxa to *Oncidiinae* used in this study available on local crops that were not previously reported in GenBank. Voucher numbers cited correspond to specimens with which our specimens were compared and validated.

Taxon	GenBank accession number	Source; locality	Voucher
Oncidiinae sp [Santanderella amaao-rinconiana]	HQ219251.1	Orlando Rincon (isotype) - Floridablanca (Santander)	P. Ortiz 1335 (HPUJ)
Macrocliniumxiphophorum	HQ219252.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz 4358 (HPUJ)
Notylia incurva	HQ219253.1	Arturo José Carrillo - Villeta (Cundinamarca)	G. Misas 214b (HPUJ)
Notyliopsisbeatricis	HQ219254.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz 1061 (HPUJ)
Oncidium (Trichocentrum) lanceanum	HQ219255.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz s.n. (HPUJ)
Oncidiumornithorhynchum	HQ219256.1	Roberto Carrascal - Bogotá D.C.	P. Ortiz 110 (HPUJ)
Oncidiumcultratum	HQ219257.1	Roberto Carrascal - Bogotá D.C.	P. Ortiz 187 (HPUJ)
Oncidium (Otoglossum) globuliferum	HQ219258.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz 54 (HPUJ)
Oncidiumfuscatum	HQ219259.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz 436 (HPUJ)
Brassia sp	HQ219260.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz 4210 (HPUJ)
Macradeniabrassavolae	HQ219250.1	Arturo José Carrillo - Villeta (Cundinamarca)	P. Ortiz 895 (HPUJ)
Trichocentrumpulchrum	HQ219261.1	Roberto Carrascal - Bogotá D.C.	P. Ortiz 702 (HPUJ)
Oliverianaortizii	HQ219262.1	Luis Eduardo Alvarez - Arcabuco (Boyacá)	P. Ortiz 101 (COL)
Telipogonnervosus	HQ219263.1	Luis Eduardo Álvarez - Guatavita (Cundinamarca)	P. Ortiz 970 (HPUJ)
Oncidium (Trichocentrum) carthagenense	HQ219264.1	SócratesForero - Silvania (Cundinamarca)	P. Ortiz 143 (HPUJ)

Table 2. Oncidiinae taxa used in the phylogenetic analysis of *matK-trnK* and *ITS*1-5.8S-*ITS*2 sequence data. N. A.: Not available.

Taxon	matK-trnK	ITS1-5.8S-ITS2
Macroclinium aurorae Whitten 3005	FJ565118.1	FJ565626.1
Macroclinium dalstroemii Whitten 2509	FJ565072.1	FJ565585.1
Macroclinium sp. Dressler 6349.	FJ565437.1	FJ564931.1
Macroclinium xiphophorum isolate P. Ortiz	HQ219252.1	JN189789
Macroclinium bicolor	AF350629.1	AF350550.1
Macroclinium robustum Gerlach 93/3019 M	FJ563935.1	FJ565344.1
Macroclinium alleniorum	EF079188.1	EF079399.1
Notylia sp. Whitten 1530	FJ564966.1	FJ565482.1
Notylia pittieri	FJ565181.1	FJ564701.1
Notylia ecuadorensis Whitten	FJ565477.1	FJ564961.1
Notylia sp. Trujillo 427	FJ564752.1	FJ565240.1
Notylia incurva isolate G. Misas 214b	HQ219253.1	JN189790
Santanderella amado-rinconiana P. Ortiz 1335 HQ219251.1	JN189792	
Notyliopsis beatricis P.Ortiz 1061	HQ219254.1	JN189791
Notylia sp. Whitten 1544	FJ564966.1	FJ565482.1
Notylia barkeri Whitten 3445	FJ565300.1	AF350624.1
Notylia albida Whitten 2823	FJ565613.1	FJ565105.1
Notylia venezuelana	EF079193.1	EF079397.1
Macradenia tridentata Hirtz 8	FJ565405.1	FJ564896.1
Macradenia rubescens Gerlach	FJ564839.1	FJ565345.1
Macradenia brassavolae Chase O-166 K	FJ563854.1	FJ565220.1
Gomesa sp. Pansarin 968	FJ564919.1	FJ565426.1
Maxillaria aciantha	DQ209876.1	DQ210296.1
Schunkea vierlingii Gerlach 0-21958 M	FJ563933.1	FJ565340.1
Warmingia eugenii Williams N192	FJ563905.1	FJ565285.1
Warmingia zamorana Hirtz 7291	FJ563944.1	FJ565369.1
Seegeriella pinifolia Gerlach 0-22556 M	FJ564829.1	FJ565339.1
Sutrina garayi Gerlach 0-22308 M	FJ564828.1	FJ565338.1
Ionopsis utricularioides Whitten 2346	FJ565042.1	FJ565557.1
Comparettia falcata Whitten 2688	FJ565090.1	FJ565601.1
Scelochilus sp. Luis Mendoza s.n.	EF079192.1	EF079394.1
Rodriguezia batemanii Whitten 1615	FJ564975.1	FJ565491.1

Table 3. *matK-trnK* forward and reverse primer sequences, fragment length sequenced, and location within *matK-trnK*.

for/rev primers	sequence	length	*matK-trnK* location
390F/ 1326R	CGATCTATTCATTCAATATTTC TCTAGCACACGAAAGTCGAAGT	936 bp	2962-3897
19F/ 556R	CGTTCTGACCATATTGCACTATG GAAGAAACATCTTTGATCCA	614 bp	2488-3101

Table 4. *ITS*1-5.8S-*ITS2* forward and reverse primer sequences, fragment length sequenced, and location within ITS.

primers	sequence	length	ITS location
17SE/ 26SE	ACGAATTCATGGTCCGGTGAAGTGTTCG TAGAATTCCCCGGTTCGCTCGCCGTTAC	724 bp	18S-26S rRNA

Table 5. Characterization of DNA sequences and parsimony analyses conducted for each molecular marker used in this study.

Marker comparisons:

			Informative sites		
Marker	bp	excl. gaps	no.	% total	% excl. gaps
ITS	724	558	44	6	7.9
matK-trnK	1436	1194	119	7.9	10
combined	2180	1611	163	13.9	17.9

Tree analyses:

Marker	Best tree length	# most parsimonious trees	Consistency index (excl. uninformative)	Retention index
ITS	179	3141	0.65	0.82
matK-trnK	412	9543	0.43	0.75
combined	749	7678	0.52	0.73